Clavulanic acid resistance: Characterization of mutants of OHIO-1 *B*-lactamase. R.A. Bonomo*, C. Dawes, J.R. Knox and D.M. Shlaes. Research Service, Dept. of Veterans Affairs Med Ctr, Dept. of Medicine, Case Western Reserve University, Cleveland, OH and Dept. Molecular Biology, U. Conn., Storrs, CT.

The OHIO-1 *B*-lactamase is a class A enzyme belonging to the SHV family. A spontaneous M69I mutant was shown to be resistant to mechanism-based inhibitors like clavulanic acid. A spontaneous G242C mutant was able to hydrolyze cefotaxime (not a substrate of the wild type) and was hyper-susceptible to inhibitors. The double mutant M69I, G242C was constructed and remained resistant to inhibitors and lost ability to bind cefotaxime. 3D modeling of the mutant enzymes with clavulanic acid and cefotaxime, using the crystal structure coordinates for the homologous *B. licheniformis B*-lactamase substituted with OHIO-1 amino acids was performed. G242C results in a displacement of the B3 strand outward, opening the lower portion of the active site to larger substrates. Some displacement of R244 on the B4 strand may also occur, but the enzyme remains hyper-susceptible to clavulanic acid. Comparison of I with M revealed that I has a similar volume but is twice as hydrophobic. The M69I mutation, lying behind the B3 and B4 strands, results in hydrophobic attraction. collapsing the active site. This also increases the distance for R244 to interact with the carboxyl of clavulanic acid. In the double mutant, the collapse of the oxyanion hole and the displacement of R244 results in an even higher Km for inhibitors. In the double mutant the collapse of the oxyanion hole clearly has more influence on cefotaxime binding than any B3 movement produced by the G242C mutation.

Enzyme	Ki Clav (uM)	Km Cefotaxime (uM)
OHIO-1	0.21	>1000
M69I	15.1	>1000
G242C	.03	45
G242C,M69I	500	>1000